



HAL
open science

Toxicity and phototoxicity of Hypocrellin A on malignant human cell lines, evidence of a synergistic action of photodynamic therapy with Imatinib mesylate

Sirinart Chio-Srichan, Noufissa Oudrhiri, Annelise Bennaceur-Griscelli, Ali G Turhan, Paul Dumas, Matthieu M. Refregiers

► To cite this version:

Sirinart Chio-Srichan, Noufissa Oudrhiri, Annelise Bennaceur-Griscelli, Ali G Turhan, Paul Dumas, et al.. Toxicity and phototoxicity of Hypocrellin A on malignant human cell lines, evidence of a synergistic action of photodynamic therapy with Imatinib mesylate. *Journal of Photochemistry and Photobiology B: Biology*, 2010, 99 (2), pp.100-104. 10.1016/j.jphotobiol.2010.03.001 . hal-01570974

HAL Id: hal-01570974

<https://hal.science/hal-01570974>

Submitted on 1 Aug 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Toxicity and Phototoxicity of Hypocrellin A on Malignant Human Cell Lines,
Evidence of a Synergistic Action of Photodynamic Therapy with Imatinib
Mesylate**

Sirinart CHIO-SRICHAN^a, Noufissa OUDRHIRI^{b-c}, Annelise BENNACEUR-GRISCELLI^{b-c}, Ali G.TURHAN^{c-d}, Paul DUMAS^a, and Matthieu REFREGIERS^{*a}

Authors affiliation:

a: Synchrotron SOLEIL, L'Orme des Merisiers, 91192 GIF SUR YVETTE, France

b: Service d'Hématologie et Biologie des tumeurs, CHU Paul Brousse APHP, Université Paris Sud 11, VILLEJUIF, France

c: INSERM U935, Paul Brousse Université Paris Sud 11, APHP, VILLEJUIF, France

d: INSERM U935, Service d'Hématologie et d'Oncologie Biologique, CHU Poitiers, et Université de Poitiers, 86021 POITIERS, France

Corresponding author : Matthieu Réfrégiers Matthieu.Refrégiers@synchrotron-soleil.fr

L'Orme des merisiers – Saint Aubin – BP48 – 91192 GIF SUR YVETTE cedex –
FRANCE

Tél : (33) 1 69359655

Fax : (33) 1 69359456

ABSTRACT

Photodynamic therapy combines a photosensitizer, localised preferentially in malignant cells with light activation. Hypocrellin A (HA), a lipid-soluble peryloquinone, is considered as a high potential photosensitizer.

We report dose and light irradiation effects of HA on HeLa, Calu and K562 cell lines, the latter including a subclone resistant to Imatinib mesylate (IM, Gleevec). All cell lines and subclones tested are sensitive to HA PDT.

In the epithelial tumour cell lines, we observe a significant photosensitizing effect in the presence of HA. In the leukemic K562 cells, HA exposure led to an inhibitory effect, which was not seen in the K562 cells resistant to Imatinib mesylate. However, experiments using IM and HA led to a reversal of IM-resistant phenotype in this cell line, with evidence of a major sensitizing effect of photodynamic therapy.

Overall our results suggest a phototoxicity of HA in epithelial cell lines and demonstrate for the first time, a synergy between IM and photodynamic therapy to circumvent IM-resistance.

Keywords: hypocrellin A– photodynamic therapy –imatinib mesylate

PACS: 87.19.xj; 82.50.Hp

Abbreviations: CML: Chronic myelogenous leukemia, HA: Hypocrellin A, IC50: Half maximal inhibitory concentration, IM: Imatinib mesylate, MTT: tetrazolium salt, PBS: Phosphate buffer saline, PDT: Photodynamic therapy, ROS: Reactive oxygen species,

1. INTRODUCTION

Photodynamic therapy (PDT) is based on the interaction of light with a photosensitizer, producing radical species that induce death of malignant cells and infectious organisms [1]. Each phenomenon taken individually has no real toxic effect while their combination leads to major damages. This therapy is very attractive, combining the chemical selectivity of the photosensitizer to the optical selectivity of the excitation light. The photodynamic effects leading to the death of cancer cells may occur through two principle pathways e.g. the production of active free radicals by electron proton transfer reaction with biomolecules (Type I) and/or the production of singlet oxygen when the photosensitizer transfers the energy to oxygen (Type II). Both pathways require light to excite the photosensitizer and also oxygen to propagate active species via radical chain reaction [2]. The double target selectivity of such treatments becomes increasingly attractive for medical applications. Since the approval of Photofrin® in the EU and the FDA (U.S.) as well as in Asian countries [3], clinical trials with PDT increased considerably including the treatment of precancerous lesions [4], of neovascular macular degeneration [5], and in the therapy of Barrett's oesophagus [6]. Its cost-utility for some clinical indications has already been assessed [7]. In several clinical trials, PDT has been shown to induce apoptosis of malignant cells, essentially in pancreatic carcinoma [8], vocal cord carcinoma [9], and esophageal carcinoma [10].

Hypocrellin A (HA), a natural hydrophobic peryloquinone derivative isolated from natural fungi sacs of *Hypocrella bambusae*, has gained considerable interest since its anticancer and antiviral activities have been reported [11, 12]. HA has been used in Chinese traditional medicine for several hundred years [13]. In vitro, numerous targets

were described for the action of photosensitized HA. Membrane damages [14] and DNA strand breakage [15] were described as possible effects induced by HA photosensitization. As for other natural perylenquinones, HA is a very hydrophobic molecule that could also target kinases [16] and low density lipoproteins [17]. The fluorescent properties of hypocrellins have been used to study cellular uptake and intracellular distribution using confocal fluorescence microscopy [18,19].

However, only few studies tested HA phototoxicity [20]; mainly on HeLa [21], S-180 [22] and MGC803 cell lines [23]. In this work, we aimed at evaluating the efficiency of HA phototoxicity at its maximal absorption wavelength on three different cell lines including HeLa (used as reference), Calu-1 (epidermoid lung carcinoma) and K562, a Philadelphia chromosome positive leukaemia cell line established from a patient with chronic myelogenous leukaemia (CML) in blast crisis. As a comparison to this latter cell line we have used also an Imatinib-mesylate-resistant K562 subclone in order to evaluate the synergy between photodynamic therapy and imatinib mesylate (STI571, Gleevec®)

2. MATERIALS AND METHODS

2.1 Chemicals. Stock solution of HA (Interchim, Montluçon, France) was dissolved in 100% DMSO at a concentration of 10^{-3} M and was stored in the dark at 4°C. Final concentration of DMSO was always kept carefully below 1 % during sample dilutions. Imatinib mesylate formerly known as STI571 or Gleevec® in USA was provided by Novartis (Rueil-Malmaison, France). All chemicals and media for cell culture were purchased from Gibco-Invitrogen, (Cergy-Pontoise, France).

2.2 Cell culture. HeLa (ATCC) and Calu-1 (ATCC) cells were routinely cultured as monolayer and were grown in Dulbecco's modified Eagle medium (DMEM) (Gibco-Invitrogen, Cergy-Pontoise, France) containing L-glutamine (862 mg/l), sodium pyruvate

(110 mg/l) and glucose (4500 mg/l), supplemented with 10% foetal calf serum (FCS), penicillin (50 µg/ml) and streptomycin (50µg/ml). Cells were maintained at 37°C in a humidified atmosphere of 5% CO₂ and were subcultured every 3-4 days. K562 and its IM-resistant subline (K562-R) were obtained from Dr Weisberg (Dana Faber Cancer Institute) cultured in RPMI-1640 containing 10% FCS. These cells were cultured as previously described, in the presence of 1 µM of imatinib mesylate [24].

2.3 HA toxicity on cells. Cell survival was estimated by MTT assays [25]. Adherent (HeLa and Calu-1) and non-adherent (K562) cells were plated into 96 well microplates at a concentration of 5 × 10⁵ cells/ml and allowed to grow for 16 hours before the treatment. Thereafter, the old medium was replaced by the fresh media mixed with HA at different concentrations (0, 0.1, 2, 10 and 20 µM). Cells were incubated with the drug for 4 hours; this incubation time was estimated as appropriate for better PDT efficiency considering intracellular localisation of the drug [18, 20]. The tetrazolium dyes used in proliferation assay have been known to interfere with some plant compounds [26]. All treatment solutions were removed from the cells prior to the addition of the MTT solution. The cells were rinsed with PBS before MTT was added. Cell survival was assessed with an absorption microplate reader (Asys UVM 340) piloting by DigiRead Software at 590 nm and the reference absorbance at 630 nm.

All cell manipulations in the presence of the photosensitizer were performed in the dark. Each experiment was performed twice independently in triplicate. Cells cultured without drugs and not irradiated were used as standard against which survival of treated and irradiated cells was compared. A negative control containing MTT reagent without cells and a positive control containing cells killed with 50 % DMSO (Sigma-Aldrich, Saint-Quentin Fallavier, France) were realised. Another parameter used as the basis for colorimetric assays is the metabolic activity of viable cells. Only metabolically active cells

reduce Tetrazolium salts [27]. Thus, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) can be reduced to a blue colored formazan [25].

2.4 Evaluation of the Photodynamic effects of HA on cell cell lines As mentioned above, after a period of incubation of 4 hours in the presence of HA, each cell line was irradiated with 2 and 10 J.cm⁻² at 460 nm with a Luxeon LED (Conrad, Lille, France). 460 nm is one of the strongest absorption bands of HA (Figure 2). Assuming that the irradiation period shall be minimised, we decided to irradiate the cells at the maximal absorption wavelength, in the blue [18], in order to decrease the irradiation time for the same effect. The remaining absorbance of HA at 590 nm may impact on formazan absorption readout and was corrected as

$$OD_{590} = 10^4 0.2 C$$

(*C* being HA concentration, 10⁴ corresponding to the molar emission coefficient of HA at 590 nm [18] and 0.2 being the optical pathway through the microplate reader) before subtraction to the final absorption read.

The half maximal inhibitory concentration (IC₅₀) of HA for every cell line and each light dose is estimated by fitting the cell toxicity assays with monoexponential decay:

$$Y = m1 + m2 \exp (-m3 C)$$

Where *Y* is the cell survival, *m1 + m2* the maximum cell survival to dark toxicity, *m1* the minimal cell survival observed and *m3* an evaluation of HA efficiency.

In order to check the relevance of the data, two way ANOVA analysis was performed on the three K562 cell populations (sensitive, resistant and resistant treated with imatinib). Two independent times three individual experiments were tested against two factors, the cell line and the light dose. The same variance analysis was performed on HeLa cells with one factor, the light dose.

The synergistic effect was assessed according to Nachbaur [28] according to the following formulas: synergistic drug interaction: surviving fraction (SF) of compounds HA and IM $< SF(HA) \times SF(IM)$.

3. RESULTS

3.1 HA phototoxicity on HeLa cells

We first evaluated the effects of HA on the phototoxicity of light as compared to dark toxicity. As can be seen in Figure 1, a slight (10 % decrease) dark toxicity at 2 μ M is observed with HeLa cells

in agreement with what was already reported on MGC803 cell line [23]. Irradiation alone, without drug, has a negligible effect. Phototoxicity is clearly dependent of the drug concentration and light doses. For HeLa cells, the IC₅₀ (Table 1, Figure 1) is 1.59 μ M of HA for a 2 J.cm⁻² irradiation and 690 nM for a 10 J.cm⁻² irradiation.

3.2 HA phototoxicity on Calu-1 cells

We next evaluated the potential synergy between HA and photodynamic toxicity in Calu-1 cell line.

Dark toxicity of HA at 2 μ M on Calu-1 is estimated around 10 % (Figure 2). As compared to non-irradiated control, Phototoxicity of HA on Calu-1 cells is extremely efficient and proportional to the doses of light and HA. The estimated IC₅₀, taking into account an exponential increase of the toxicity, is 760 nM at 2 J.cm⁻² and 460 nM at 10 J.cm⁻².

3.3 HA phototoxicity on K562 cells

K562 cells are non-adherent hematopoietic cells and we have first evaluated if the concentrations of HA used in the HeLa and Calu-1 cell lines were not toxic for the K562

cells. We then performed phototoxicity experiments in the presence or in the absence of these concentrations of HA. As can be seen in Figure 3, K562 cells present a strong dark toxicity around 20 % (80% cell survival) at several concentrations of HA. No light toxicity is observed and the phototoxicity at 2 J.cm⁻² and 10 J.cm⁻² are rather strong with IC₅₀ of respectively 1.2 μM and 100 nM (Figure 3 and table 1).

3.4 HA phototoxicity on K562 RSTI571 cells

We then evaluated the impact of the phototoxicity of HA on the IM-resistant counterparts of K562 cells. The resistance to IM seems to impact also on HA dark toxicity as none is observed at 2μM and less than 20 % up to 20 μM. However, in presence of 460 nm light, the cell survival decreases dramatically (Figure 4 and table 1). IC₅₀ have been found to be 1.69 μM at 2 J.cm⁻² and 590 nM at 10 J.cm⁻².

3.5 Evaluation of a synergistic effects of HA and IM on K562 R cells

In order to determine if a synergistic effect between IM and HA, IM-resistant cells were treated with 1 μM of imatinib mesylate and increasing doses of HA. These experiments showed a very significant combined effect on dark toxicity (Figure 5), from 10 % at 2μM HA up to 30 % at 20 μM.

At 2J.cm⁻² SF(imatinib) x SF(HA) = 0.42 x 0.5 = 0.21 for 1.68 μM of HA and 1μM of imatinib

At 10 J.cm⁻² SF(imatinib) x SF(HA) = 0.42 x 0.5 = 0.21 for 590 nM of HA and 1 μM of imatinib

The respective SF(imatinib+HA) are 22% at 2J.cm⁻² and 5 % at 10J.cm⁻².

This demonstrates a synergistic effect at 10J.cm⁻² that neither imatinib mesylate at 1μM (Figure 5) nor HA at 20 μM alone exhibit separately (Figure 4) on K562 RSTI571 cells.

The estimated IC₅₀ for the combination are 840 mM at 2 J.cm⁻² and 180 nM at 10 J.cm⁻².

4. DISCUSSION

In this study, we have evaluated the effects of the photodynamic therapy in human malignant cell lines and we explored its potential synergy with Imatinib mesylate in K562 cells expressing the BCR-ABL oncogene. Toxicity assays were performed for evaluating the dark toxicity and phototoxicity of HA on the four cell lines. In addition, the influence of the combination of the drug concentration and of the light dose delivery on the cells proliferation was investigated.

In the first part of the study, we have performed a comparative analysis of the phototoxicity of HA on different cell lines (Table 1).

The phototoxicity of HA on Calu-1 is almost twice as the observed phototoxicity on HeLa. The high sensitivity of Calu-1 to HA PDT demonstrate that this carcinoma cell line, being located in lung, is a good candidate for PDT.

The results obtained by the use of K562 cells and their IM-resistant subclone show for the first time to our knowledge, that K562 cells and their IM-resistant subclone are very sensitive to HA PDT. Despite the fact that K562-resistant cells have slightly higher IC50 as compared to parental K562 cells (Table 1), the observed difference for resistant cells in the dark and in the presence of light (Figure 4) suggest that HA have a cytotoxic effect in this setting, which requires further evaluation. CML is a model of targeted therapy of cancer in which the majority of leukemic cells can effectively be killed by IM therapy whereas the more primitive malignant stem cells are resistant to therapy [29]. Several mechanisms might be involved in this resistance, including high levels of Bcr-ABL expression in primitive stem cells [30]. Primitive CML stem cells have also been shown to harbour ABL-kinase mutations, interfering with the efficient binding of IM to its target [31].

We then asked whether there could be a synergistic effect between the HA and Imatinib mesylate in IM-resistant K562 cells. As can be seen in Figure 5, there is a significant synergy between HA and IM, especially with higher doses of light exposure (Table 1 and Figure 5). As probed by variance analysis, the two main discriminants ($P < 0.01$) are the light dose and the cell population (Table 2). Cell population (respectively K562, K562-resistant and K562-resistant with imatinib treatment) is found to be highly discriminatory. Imatinib mesylate and photodynamic therapy combined effect (Figure 5 and table 1) is close to the synergy already observed in K562-R cells treated [32] for imatinib resistant cell lines. The potential mechanism of this synergy could be the combined antikinase activity of perylenequinones [16] and the antiTK activity of Imatinib mesylate. Another mechanism explaining the synergy with HA in K562 cells could be the fact that Bcr-ABL overexpression generates high levels of ROS in leukemic cells balanced by the induced resistance to apoptosis. This equilibrium could be shifted by the overproduction of ROS generated via PDT. Imatinib combination therapy seems to overcome resistance for in vitro studies and trials of combination with other molecules [33-34].

One interesting challenging question is to evaluate if this synergistic activity exist at the level of leukemic hematopoietic stem cells that are intrinsically resistant to Imatinib mesylate [29]. Combination of PDT with imatinib could therefore be explored as an experimental tool for purging approaches in CML as Imatinib has already been tested for such an experimental approach [35-36]. Further experiments are needed to determine the feasibility and toxicity of this strategy in primary leukemic cells and more importantly, to establish the efficiency of this strategy at the level of Ph1+ stem cells.

Mis en forme : Anglais (États-Unis)

Acknowledgements--Royal Thai government scholarship from the ministry of science and technology, Thailand is gratefully acknowledged by the first author. We would like to thank Frédéric Jamme and Christophe Sandt for fruitful discussions.

References

- [1] M.R. Hamblin, T. Hasan, Photodynamic therapy: a new antimicrobial approach to infectious disease?, *Photochem Photobiol Sci* 3 (2004) 436-450.
- [2] M. Oschner, Photophysical and photobiological processes in the photodynamic therapy of tumours, *J Photochem Photobiol B* 39 (1997) 1-18.
- [3] M.G. Vicente, Porphyrin-based sensitizers in the detection and treatment of cancer: recent progress, *Curr Med Chem Anticancer Agents* 1 (2001) 175-194.
- [4] E.H. Tschen, D.S. Wong, D.M. Pariser, F.E. Dunlap, A. Houlihan, M.B. Ferdon, Photodynamic therapy using aminolaevulinic acid for patients with nonhyperkeratotic actinic keratoses of the face and scalp: phase IV multicentre clinical trial with 12-month follow up, *Br J Dermatol* 155 (2006) 1262-1269.
- [5] P.J. Rosenfeld, R.M. Rich, G.A. Lalwani, Ranibizumab: Phase III clinical trial results, *Ophthalmol Clin North Am* 19 (2006) 361-372.
- [6] B. Overholt, C. Lightdale, K. Wang, M. Canto, S. Burdick, R. Haggitt, M. Bronner, S. Taylor, M. Grace, M. Depot, Photodynamic therapy with porfimer sodium for ablation of high-grade dysplasia in Barrett's esophagus: international, partially blinded, randomized phase III trial, *Gastrointest Endosc* 62 (2005) 488-498.
- [7] G.C. Brown, M.M. Brown, J. Campanella, G.R. Beauchamp, The cost-utility of photodynamic therapy in eyes with neovascular macular degeneration – a value- based reappraisal with 5-year data, *Am J Ophthalmol* 140 (2005) 679-687.
- [8] L. Ayaru, S. G. Bown, S. P. Pereira, Photodynamic therapy for pancreatic carcinoma: experimental and clinical studies, *Photodiagnosis Photodyn Ther* 1 (2004) 145-155.
- [9] C. Freche, S. De Corbiere, Use of photodynamic therapy in the treatment of vocal cord carcinoma, *J Photochem Photobiol B* 6 (1990) 291-296.
- [10] A. Maier, F. Tomaselli, V. Matzi, P. Rehak, H. Pinter, F.M. Smolle-Jüttner,

- Photosensitization with hematoporphyrin derivative compared to 5-aminolaevulinic acid for photodynamic therapy of esophageal carcinoma, *Ann Thorac Surg* 72 (2001) 1136-1140.
- [11] Z. Diwu, Novel therapeutic and diagnostic applications of hypocrellins and hypericins, *Photochem Photobiol* 61 (1995) 529-539.
- [12] S.M. Ali, S.K. Chee, G.Y. Yuen, M. Olivo, Hypericin and hypocrellin induced apoptosis in human mucosal carcinoma cells, *J Photochem Photobiol B* 65 (2001) 59-73.
- [13] D. Zhenjun, J.W. Lown, Hypocrellins and their use in photosensitization, *Photochem Photobiol* 52 (1990) 609-616.
- [14] L.S. Cheng, J.Z. Wang, S.M. Fu, Effect of hypocrellin A sensitization on the lateral mobility of cell membrane proteins, *J Photochem Photobiol B* 2 (1988) 395-398.
- [15] E.H. Cao, S.M. Xin, L.S. Cheng, DNA damage induced by hypocrellin-A photosensitization, *Int J Radiat Biol* 61 (1992) 213-219.
- [16] S. Kocanova, T. Hornakova, J. Hritz, D. Jancura, D. Chorvat Jr, A. Mateasik, J. Ulicny, M. Refregiers, J-C. Maurizot, P. Miskovsky, Characterization of the Interaction of Hypericin with Protein Kinase C in U-87 MG Human Glioma Cells, *Photochem Photobiol* 82 (2006) 720-728.
- [17] S. Kascakova S, M. Refregiers, D. Jancura, F. Sureau, J-C. Maurizot, P. Miskovsky, Fluorescence spectroscopic study of hypericin-photosensitized oxidation of low-density lipoproteins, *Photochem Photobiol* 81 (2007) 1395-1403.
- [18] S. Chio-Srichan, M. Refregiers, F. Jamme, V. Rouam, P. Dumas, Photosensitizer effects on cancerous cells: A combined study using synchrotron infrared and fluorescence microscopies, *Biochim Biophys Acta* 5 (2008) 854-860.
- [19] G.G. Miller, K. Brown, R.B. Moore, Z.J. Diwu, J. Liu, L. Huang, J.W. Lown, D.A. Begg, V. Chlumecky, J. Tulip, M.S. McPhee, Uptake kinetics and intracellular localisation of

- Hypocrellin photosensitizers for photodynamic therapy: a confocal microscopy study, *Photochem Photobiol* 61 (1995) 632-638.
- [20] S.M. Ali, M. Olivo, Efficacy of hypocrellin pharmacokinetics in phototherapy, *Int J Oncol* 2 (2002) 1229-1237.
- [21] E. Cao, L. Cheng, DNA single-strand breakage and its rejoining in HeLa cells caused by Hypocrellin A photosensitization, *Acta Biol Exp Sin* 21 (1988) 79-85.
- [22] C. Dong, H. Jia, L. Zhang, C. Ma, The inhibitory effect of the new photosensitizer hypocrellin A on experimental tumors, *Sheng Wu Hua Hsueh Tsa Chih* 3 (1987) 468-472.
- [23] J. Zhang, E.H. Cao, J.F. Li, T.C. Zhang, W.J. Ma, Photodynamic effects of hypocrellin A on three human malignant cell lines by inducing apoptosis cell death, *Photochem Photobiol* 43 (1998) 106-111.
- [24] E. Deutsch, L. Maggiorella, B. Wen, M.L. Bonnet, K. Khanfir, V. Frascogna, A.G. Turhan, J. Bourhis, Tyrosine kinase inhibitor AG1024 exerts antileukaemic effects on STI571-resistant Bcr-Abl expressing cells and decreases AKT phosphorylation, *Br J Cancer* 91 (2004) 1735-1741.
- [25] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *J Immunol Methods* 65 (1983) 55-63.
- [26] R. Bruggisser, K. von Daeniken, G. Jundt, W. Schaffner, H. Tullberg-Reinert, Interference of plant extracts, phytoestrogens and antioxidants with the MTT tetrazolium assay, *Planta Med* 68 (2002) 445-448.
- [27] F.E. Smith, Tetrazolium salts, *Science* (1951) 751-754.
- [28] D.M. Nachbaur, H.A. Denz, G. Gastl, J. Thaler, M. Lechleitner and H. Braunsteiner, Combination effects of human recombinant interferon (alpha-2-arg, gamma) and cytotoxic agents on colony formation of human melanoma and hypernephroma cell lines, *Cancer Lett.* 44 (1989) 49-53.

- [29] X.Y. Jiang, Y. Zhao, C. Smith, M. Gasparetto, K.M. Saw, A.G. Turhan, A. Eaves, C. Eaves, Chronic myeloid leukemia stem cells possess unique properties that predict intrinsic and acquired resistance to imatinib mesylate, *Leukemia* 21 (2007) 926-935.
- [30] A. Ossard-Receveur, A. Bernheim, B. Clausse, G. Danglot, D. Fauvet, B. Leon, F. Lozach, D. Bories, C. Brouzes, J.H. Bourhis, A.G. Turhan, Duplication of the Ph-chromosome as a possible mechanism of resistance to imatinib mesylate in patients with chronic myelogenous leukemia, *Cancer Genet Cytogenet* 163 (2005) 189-190.
- [31] N. Sorel, M.L. Bonnet, M. Guillier, F. Guilhot, A. Brizard, A.G. Turhan, Evidence of ABL-kinase domain mutations in highly purified primitive stem cell populations of patients with chronic myelogenous leukemia, *Biochem Biophys Res Commun* 323 (2004) 728-730.
- [32] A.J. Tipping, F.X. Mahon, G. Zafirides, V. Lagarde, J.M. Goldman, J.V. Melo, Drug responses of imatinib mesylate-resistant cells: synergism of imatinib with other chemotherapeutic drugs, *Leukemia* 16 (2002) 2349-2357.
- [33] S.C. Paterson, K.D. Smith, T.L. Holyoake, H.G. Jørgensen, Is there a cloud in the silver lining for imatinib?, *Br J Cancer* 88 (2003) 983-987.
- [34] D.M. Ross, T.P. Hughes, Cancer treatment with kinase inhibitors: what have we learnt from imatinib? *Br J Cancer* 90 (2004) 12-19.
- [35] R. Edelson, C. Berger, F. Gasparro, B. Jegasothy, P. Heald, B. Wintroub, E. Vonderheid, R. Knobler, K. Wolff, G. Plewig, G. Mc Kierman, I. Christensen, M. Oster, H. Hönigsmann, H. Wilford, E. Kokoschka, T. Rehle, M.I. Perez, G. Stingl, L. Laroche, Treatment of cutaneous T-cell lymphoma by extracorporeal photochemotherapy. Preliminary results, *N Engl J Med* 316 (1987) 297-303.
- [36] H. Yang, C. Eaves, M. de Lima, M.S. Lee, R.E. Champlin, J.D. McMannis, S.N. Robinson, T. Niu, W.K. Decker, D. Xing, J. Ng, S. Li, X. Yao, A.C. Eaves, R. Jones, B.S. Andersson, E.J. Shpall, A novel triple purge strategy for eliminating chronic myelogenous leukemia

(CML) cells from autografts. *Bone Marrow Transplant*. 37 (2006) 575-82.

FIGURE LEGENDS

Figure 1 Toxicity and Phototoxicity of HA on HeLa cells with increasing HA concentration with no irradiation (cross), 2 J.cm⁻² irradiation (open circle) and 10 J.cm⁻² irradiation (black circle). Experiments with irradiation were fitted with first order exponential decays. The calculated IC 50 at 2 and J.cm⁻² are respectively 1.59 +/- 0.2 and 0.69 +/- 0.2 µM.

Figure 2 Toxicity and Phototoxicity of HA on Calu-1 cells with increasing HA concentration with no irradiation (cross), 2 J.cm⁻² irradiation (open circle) and 10 J.cm⁻² irradiation (black circle). Irradiated experiments were fitted with first order exponential decays. The calculated IC 50 at 2 and 10 J.cm⁻² are respectively 0.76 +/- 0.08 and 0.46 +/- 0.05 µM.

Figure 3 Toxicity and Phototoxicity of HA on K562 cells with increasing HA concentration with no irradiation (cross), 2 J.cm⁻² irradiation (open circle) and 10 J.cm⁻² irradiation (black circle). Irradiated experiments were fitted with first order exponential decays. The calculated IC 50 at 2 and 10 J.cm⁻² are respectively 1.2 +/- 0.2 and 0.1 +/- 0.01 µM.

Figure 4 Toxicity and Phototoxicity of HA on K562 RSTI571 cells with increasing HA concentration with no irradiation (cross), 2 J.cm⁻² irradiation (open circle) and 10 J.cm⁻² irradiation (black circle). Irradiated experiments were fitted with first order exponential decays. The calculated IC 50 at 2 and 10 J.cm⁻² are respectively 1.69 +/- 0.2 and 0.59 +/- 0.05 µM.

Figure 5 Toxicity and Phototoxicity of HA on K562 RSTI571 cells treated with 1 μM of imatinib and with increasing HA concentration with no irradiation (cross), 2 $\text{J}\cdot\text{cm}^{-2}$ irradiation (open circle) and 10 $\text{J}\cdot\text{cm}^{-2}$ irradiation (black circle). Irradiated experiments were fitted with first order exponential decays. The calculated IC 50 at 2 and 10 $\text{J}\cdot\text{cm}^{-2}$ are respectively 0.84 \pm 0.02 and 0.18 \pm 0.05 μM .